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The effect of a uniform static electromagnetic field on chondrocyte morphology *in vitro*

Introduction

As Bioengineering expands, tissue engineering has become an increasingly important field. A particular area which has received much attention is cartilage tissue engineering, which aims to produce bio-friendly replacements for cartilage tissue in the body. Generally, a scaffold with similar properties to collagen (as in natural cartiladge) is seeded with the cells that compose cartilaginous tissue, called chondrocytes. Most experiments focus on chemical and physical stimuli to coax the scaffolding into a tissue-like construct (Tuli, 2003). In our study, however, we investigated the effects of a uniform static electromagnetic field (SEMF) on the morphology bovine chondrocytes. In particular, we looked at the effects of a moderately powerful field on cell conformation in low-viscosity 2% alginate gel beads (Sigma Aldrich) by looking at collagen I and collagen II production and cell morphology.

Our setup was created to produce an electrically-induced uniform SEMF through the cells. As seen in Fig. 1, the setup was constructed as two aligned circular Helmholtz coils of radius 50 mm with the cells located 30 mm below the top coil and 30 mm above the bottom coil. Each coil had 12 turns (or n=12).A 6A DC current was run constantly through the system, generating a field strength of 13 milliTesla (mT). The system was set up inside of a 37^{o} incubator to allow the cells to grow. Initially, each sample began with 250,000 chondrocytes.

Results and Interpretation of Data

Initial viability check

The experimental cells were allowed to grow in the SEMF for one week; the control cells were not exposed to any created magnetic fields. Initial cell viability results indicated that most cells in both experimental and control samples were alive; interestingly, the experimental samples showed higher cell concentration and distribution than the control samples. In experimental, the cells were evenly spread out throughout the bead; however, in the control sample, cells were clustered into small colonies and were more sparsely allocated throughout the bead. In both samples cells exhibited a spherical configuration, indicated that they had probably not started to de-differentiate into fibroblasts.

RNA content analysis for collagen I/II via RT-PCR

Next, the cells were lysed to extract the RNA for RT-PCR analysis. The A_{260}/A_{280} values for a 1:100 dilution of experimental and control RNA were 0.75 and 0.5 respectively, indicating that the RNA samples were likely impure due to high protein content. For RT-PCR, 4 ng/µL of control RNA and 12 ng/µL of experimental RNA were put into a 1.2% agarose gel and run for 45 minutes at 120v. As seen in Fig. 2, the results for collagen I were very likely very low or zero for both control and experimental, as no strong bands were seen in Lanes 2 and 3. The smearing from the GAPDH housekeeping gene bands in both lanes also made it very difficult to discern any band structure at the collagen I location, indicated by the green box. For collagen II, however, the bands were very slightly visible (shown in the red box in Fig. 2). The experimental sample showed a higher amount of collagen II, with an intensity ratio of collagen II to GAPDH normalized against area of 0.055, while the control sample had a ratio of 0.050. This indicates that the experimental cells potentially maintained chondrocyte morphology better than the control cells.

Collagen I/II content analysis via indirect ELISA

The collagen I and II contents in both the experimental and control samples were investigated using indirect ELISA. Each type of collagen was assayed in a separate 96-well plate, as in Supplemental Fig. 3A (supplemental); standards were placed in the first two columns, while the experimental and control samples were placed in the third. The standardizations exhibited a strong linear behavior, as seen in Supplemental Fig. 3B. In determining the standard curves, however, the duplicate standardization columns had some variability, indicating that the resulting protein concentrations should be taken with some caution. The collagen I results for control and experimental showed a significant amount of difference, with concentrations of 65 ng/mL and 12 ng/mL, respectively. For collagen II, however, the control sample had too low an amount to measure, but the experimental sample showed a concentration of 50ng/mL. The ratio for collagen II/collagen I in the experimental sample was 4.2, indicating that a much larger amount of collagen II was present. Thus, the experimental sample showed strong chondrocyte morphology with high values of collagen II and low values of collagen I. However, the control sample seemed to show fibroblast morphology with a high concentration of collagen I and very low concentration of collagen II. This indicates that the experimental cells most likely preserved chondrocyte morphology better than the control cells, as in the RT-PCR results above.

Contextualizing Results and Suggestions for Future Work

The results above all indicated that a SEMF, even at a moderate strength of 13 mT, did have a positive effect on maintaining chondrocyte morphology in comparison to a control sample. However, many of the results had very small amounts of sample to examine, such as the amount and purity of the RNA for RT-PCR, which could introduce some inconclusive results. By using qPCR, one can keep better track of the amount of cDNA in each cycle, and thus obtain more accurate results. Similarly, using

sandwich ELISA would most likely have given more precise results for the protein assays. However, our results did indicate with some certainty that magnetic fields had a positive effect on chondrocyte morphology over 7 days. Jahns, et al. discussed the effects of a pulsed electromagnetic field (PEMF) on the chondrocyte, stating that the resulting preservation of chondrocyte phenotype could be due to the EMF effects on the calcium/calmodulin pathway (Jahns, 2007). Other studies, such as those by Blumenthal et al., indicated that DC SEMFs could affect the transmembrane signaling of cells by altering specific membrane functions (Blumenthal, 1997). SEMFs were shown to slowly realign the diamagnetic molecular domains of cell membranes, thus changing cell morphology to a spherical appearance, similar to work by Chionna et al. with Hep G2 cells (Chionna, 2005). Thus, SEMFs can potentially be a relatively simple way to safely stimulate cells in an artificial cartilage construct non-invasively to maintain and promote chondrocyte morphology. This could have important applications in actually maintaining phenotype in an implant, as the magnetic field could simply be run through the body without actual invasive surgery or any kind of chemical stimuli. It also would allow for long-term culturing of chondrocytes *in vitro* using scaffolds, as the cells would be much more likely to stay chondrocytes than to de-differentiate into fibroblasts. This experiment could be expanded upon by using PEMFs with different frequencies, and observing the differences between an SEMF and a PEMF on chondrocyte morphology. Such an experiment would provide a better insight into how magnetic fields affect cells, and whether cells can adapt over time to an SEMF and lose any benefits introduced by the magnetic field. This information could prove very important to creating very effective and safe non-invasive tissue engineering methods. PEMFs have also been implicated in repair of cartilage, and it would be very interesting to perform an *in vivo* study on the effects of SEMFs on aging cartilage to stimulate repair, perhaps as a way to treat rheumatoid arthritis (Aaron, 2006).

Works Cited

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